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Part I

SECTION A

Volume 23

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## CONTENTS

A Study of the Influence of Concentrations of Sucrose and Ammonium Nitrate in the Cultures of Dhar Yeast on Its Metabolism at Constant Carbon-Nitrogen Ratio 45, under Non-Aerated Conditions . . . . .	<i>Krishna Bahadur</i>	1
A Comparative Study of the Growth of Dhar Yeast and <i>Saccharomyces calbergensis</i> in Cultures, Containing only Ethyl Alcohol as Source of Carbon under Non-Aerated Conditions . . . . .	<i>Krishna Bahadur</i>	8
Study of the Mutations in Dhar Yeast Effected by Fuchsin Red . . . . .	<i>Krishna Bahadur</i>	13
A Study of the Influence of Hydrogen-Ion Concentration on the Synthesis of Amino Acids from Paraformaldehyde and Potassium Nitrate . . . . .	<i>S. Ranganayaki and Krishna Bahadur</i>	21
Carbon-Halogen Bond Energy in Monohalides of Normal Paraffins . . . . .	<i>Shashanka Shekhar Mitra</i>	25
The Azimuthal Effect of Cosmic Rays at Gulmarg . . . . .	<i>T. H. Naqvi</i>	30
The Composition and Quality of Maize Grains as Affected by Varieties, Manures and Spacings . . . . .	<i>R. R. Agarwal and R. N. Verma</i>	35
On a Sequence of Fourier Coefficients . . . . .	<i>Basudeo Singh</i>	42
Study in the Precipitation of Yellow and Brown Hydrous Ferric Oxide. Part XI. Electron Diffraction Study of the Different Samples of Hydrous Ferric Oxide . . . . .	<i>Sita Ram Gupta and Satyeshwar Ghosh</i>	44
Studies in the Change of the Hydrogen-Ion Concentration of Colloidal Hydrous Oxide Sols during Their Coagulation. Part I. Coagulation of Ferric Oxide Sols by Potassium Sulphate . . . . .	<i>Rama Shanker Rai and Satyeshwar Ghosh</i>	48

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A STUDY OF THE INFLUENCE OF CONCENTRA-  
TIONS OF SUCROSE AND AMMONIUM NITRATE  
IN THE CULTURES OF DHAR YEAST ON ITS  
METABOLISM AT CONSTANT CARBON-  
NITROGEN RATIO 45, UNDER NON-  
AERATED CONDITIONS

BY KRISHNA BAHADUR

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(Communicated by Dr. S. P. Mitra)

INTRODUCTION

THE influence of the concentration of carbon food in the cultures of micro-organisms has been studied by several persons. While mentioning the effect of concentration on the size of the organism Henrici<sup>1</sup> has observed great increase in size of yeast by the increase of concentration of carbon food in the media. A factor contributing to the value of a compound for growth is the degree of dilution at which it is operative, which in its turn depends on the affinity of the substance for its enzyme. Glucose has been found to have high affinity as compared to other carbohydrates. Friedlein<sup>2</sup> determined the minimum concentration of certain compounds at which growth can occur.

The rate of growth in the logarithmic phase is largely independent of concentration of carbon nutrients until this reaches a low level.

The relation of total crop to concentration of medium has been tested out by Monod,<sup>3</sup> using coli, growing on brain broth and the total crop was shown to be strictly proportional to the concentration of the medium between 0.005 and 0.1 g./ml. (wet weight). No work has been done so far to elucidate the influence of the concentration of the carbon and nitrogen food on the other physiological properties of an organism.

It is fully established that there is a loss of more than 50 per cent. of nitrogen when mineral nitrogenous compounds are added to the soil for improving crop production.<sup>4</sup> The carbon-nitrogen ratio of the soil plays an important role in this loss, which is minimised by increase of carbon-nitrogen ratio of the soil. I have studied the loss of nitrogen in Dhar Yeast<sup>5</sup> cultures at different carbon-nitrogen ratios and have found a similar loss of nitrogen in the yeast cultures also. In this paper I have given the results obtained by the study of the influence of concentration of carbon and nitrogenous substances on the loss of nitrogen and other metabolic activities at constant carbon-nitrogen ratio of 45. The variation of the concentration of carbon was achieved by changing the concentration of sucrose, the sole source of carbon in these cultures, from 0.5 to 2.0 per cent. Ammonium nitrate has been used as the source of nitrogen in the cultures and its amount was changed accordingly to give carbon-nitrogen ratio 45.

#### EXPERIMENTAL

Four cultures each containing 0.1 g. of calcium carbonate, 0.125 g. of magnesium carbonate, 0.1 g. of sodium chloride, 0.1 g. of potassium sulphate and 0.1 g. of disodium hydrogen phosphate were prepared. In each of them amounts of sucrose and ammonium nitrate as mentioned in Table I below were added. These substances were digested with dilute hydrochloric acid and kept in 500 c.c. flat-bottom pyrex flasks. The total volume of each culture was made to 200 c.c. and their pH was adjusted to be 4.5. The flasks were cotton-plugged with non-absorbent cotton and sterilised by heating at 15 lb. pressure for 30 minutes in an autoclave. After cooling these cultures were kept together at room temperature which varied during the period of fermentation between 29.7° to 32.0° C. The cultures were analysed after 26 days.

Estimation of nitrogen in these cultures was done by Kjeldahl and Gunning method.<sup>6-10</sup> To estimate the amount of nitrogen left in the culture 100 c.c. of the clear solution obtained after filtering off the yeast were dried to almost dryness in a Kjeldahl flask. To the residue were added 5 g. of potassium sulphate, 1 g. of salicylic acid, 0.2 g. of copper sulphate and 20 c.c. of pure concentrated sulphuric acid and the mixture was boiled

till it became clear. This clear solution was distilled in presence of excess of alkali and the ammonia thus set free was estimated by absorbing it in a standard acid solution. This acid was then titrated with standard alkali and the amount of ammonia, and so the nitrogen present in the original solution, was calculated.

To estimate the nitrogen in yeast cells known weight of yeast, dried at 60° C., was digested with concentrated sulphuric acid in presence of copper sulphate and potassium sulphate. And then the amount of nitrogen was estimated as mentioned above.

All the compounds used in the preparation of cultures and analysis were of A.R. quality.

#### OBSERVATION

Results obtained by the analysis of the above cultures are tabulated below.

TABLE I  
*Sugar Data*

Serial No.	g. of sucrose added in the culture before fermentation	g. of ammonium nitrate used in the culture as nitrogen food in the beginning	g. of total sugar left in the culture after fermentation	g. of reducing sugar left	g. of non-reducing sugar left	g. of sugar consumed during fermentation	Percentage of sugar consumed calculated on the basis of original sugar
1	1.00	0.0272	0.75	0.73	0.02	0.25	25.00
2	2.00	0.0543	0.76	0.72	0.04	1.24	82.00
3	3.00	0.0814	0.55	0.54	0.01	2.45	88.16
4	4.00	0.1086	1.50	0.92	0.58	2.50	62.50

TABLE II  
*Acid Formation*

Serial No.	g. of sucrose added in the culture as carbon food	g. of ammonium nitrate added in the culture as nitrogen food	g. of total acid formed during fermentation	g. of volatile acid produced	g. of non-volatile acid formed	Percentage of acid formation calculated on the basis of sugar consumed
1	1.00	0.0272	0.1344	0.0005	0.1339	53.76
2	2.00	0.0543	0.1972	0.0005	0.1967	18.87
3	3.00	0.0814	0.1953	0.0009	0.1944	7.90
4	4.00	0.1086	0.2028	0.0009	0.2018	8.11

TABLE III  
*Yeast Growth and Alcohol Formation*

Serial No.	g. of sucrose added in the culture as carbon food	g. of ammonium nitrate added in the culture as nitrogen food	g. of dry yeast grown during fermentation	Percentage of yeast yield calculated on the basis of sugar consumed	g. of ethyl alcohol formed during fermentation	Percentage of alcohol formation calculated on the basis of sugar consumed
1	1.00	0.0272	0.0786	31.44	0.00	0.00
2	2.00	0.0543	0.1930	15.56	0.00	0.00
3	3.00	0.0814	0.2414	9.05	0.00	0.00
4	4.00	0.1086	0.3094	12.37	0.00	0.00

TABLE IV  
*Carbon Data*

Serial No.	g. of sucrose in the culture in the beginning	g. of ammonium nitrate in the culture in the beginning	g. of carbon consumed during fermentation	g. of carbon gone in the yeast cells grown during fermentation	Percentage of carbon utilised in the yeast growth calculated on the basis of carbon consumed	Percentage of carbon in the yeast cells
1	1.00	0.0272	0.0673	0.0179	26.58	22.07
2	2.00	0.0543	0.3647	0.0441	12.08	22.85
3	3.00	0.0814	0.7200	0.0553	7.68	33.52
4	4.00	0.1086	0.7347	0.0707	9.63	43.76

TABLE V  
*Nitrogen Data*

Serial No.	g. of sucrose in the culture in the beginning	g. of ammonium nitrate in the culture in the beginning	g. of nitrogen present in the culture before fermentation	g. of ammoniacal nitrogen in the beginning	g. of nitric nitrogen in the beginning	g. of total nitrogen left in the culture after fermentation	g. of ammoniacal nitrogen left in the culture
1	1.00	0.0272	0.0095	0.0047	0.0047	0.0024	0.0000
2	2.00	0.0543	0.0190	0.0095	0.0095	0.0087	0.0000
3	3.00	0.0814	0.0286	0.0142	0.0142	0.01617	0.0024
4	4.00	0.1086	0.0380	0.0190	0.0190	0.0229	0.0233

TABLE VI  
*Nitrogen Data—(Continued)*

Serial No.	g. of nitric nitrogen left in the culture	g. of total nitrogen consumed	g. of ammoniacal nitrogen consumed	g. of nitric nitrogen consumed	Percentage of ammoniacal nitrogen utilised calculated on the basis of total nitrogen consumed	Percentage of nitric nitrogen utilised calculated on the basis of total nitrogen consumed
1	0.0024	0.0071	0.0047	0.0023	66.18	32.39
2	0.0087	0.0102	0.0095	0.0007	92.57	7.40
3	0.0135	0.0124	0.0118	0.0006	95.84	5.02
4	0.0376	0.0150	0.0147	0.0003	97.54	2.50

TABLE VII  
*Nitrogen Data—(Continued)*

Serial No.	g. of nitrogen gone in the yeast cells grown during fermentation	g. of nitrogen lost during the period of fermentation	Percentage of loss of nitrogen calculated on the basis of nitrogen consumed	g. of dry yeast grown by the consumption of 1 g. of nitrogen	Percentage of nitrogen in the yeast cells
1	0.0038	0.0033	46.47	18.71	4.83
2	0.0097	0.0005	4.96	18.08	5.02
3	0.0121	0.0002	1.94	19.42	5.01
4	0.0146	0.0004	0.26	19.82	4.71

#### INFERENCE

Increase of the concentration of sucrose and ammonium nitrate in the yeast cultures at a constant carbon-nitrogen ratio of 45 increases the sugar consumption of Dhar Yeast upto 1.5 per cent. concentration of sucrose in the culture, after which the sugar consuming property of the yeast decreases with the increase of concentration. Acid formation decreases with the increase of concentration in the culture.

Grams of yeast grown during the period of fermentation increases with the increase of concentration in the culture. But the percentage of yeast yield calculated on the basis of sugar consumed decreases with the increase of concentration in the culture upto 1.5 per cent. of sugar in the culture. After this limit the increase of concentration increases this percentage of yeast yield.

As the concentration increases in the culture lesser percentage of the carbon burnt during the fermentation is utilised in being converted into the carbon of the yeast cell. Thus out of a total of 0.0673 g. of carbon consumed during the period of fermentation, in the culture containing 0.5 per cent. of sucrose, 26.58 per cent. goes in the yeast cells grown during that period. But out of 0.7347 g. of carbon consumed during fermentation in the culture containing 2.0 per cent. of sucrose, only 9.62 per cent. goes in the yeast cells.

The percentage of carbon in the yeast cells increases with the increase of concentration in the culture. It being 22.7 per cent. in the culture containing 0.5 per cent. of sucrose and 43.76 per cent. when the concentration of sucrose is 2 per cent.

Percentage of ammoniacal nitrogen consumed during fermentation calculated on the basis of total nitrogen consumed, increases with the increase of concentration in the culture. But the percentage of nitric nitrogen consumed during this process decreases with the increase of concentration. Thus 32.39 per cent. of the total nitrogen consumed during fermentation is nitric-nitrogen when the concentration of sucrose is 0.5 per cent. in the culture. It decreases with the increase of concentration and reaches to 2.5 per cent. when concentration of sucrose in the culture increases to 2 per cent.

The loss of nitrogen of the yeast cultures is profoundly influenced by the concentration. This loss rapidly decreases with the increase of concentration. It being 46.47 per cent. at 0.5 per cent. sucrose concentration in the medium and only 0.26 per cent. in the culture containing 2.0 per cent. of sucrose.

#### SUMMARY

Increase of the concentration of sucrose and ammonium nitrate in Dhar Yeast cultures, keeping the carbon-nitrogen ratio of the culture constant at 45, increases sugar consumption, and growth of yeast in grams grown in the culture, and decreases acid formation and yeast yield calculated on the basis of sugar consumed. The percentage of carbon utilised in the formation of yeast cells, calculated on the basis of total carbon consumed, decreases with the increase of concentration in the culture. The percentage of carbon in the yeast increases as the concentration increases.

The above facts indicate that the increase of concentration increases the respiration rate of the yeast. A greater consumption of sugar with lesser amount of this consumed carbon being utilised in the formation of yeast cells and this being not followed by any increase in acid, alcohol or any carbonaceous substance formation, proves that at higher concentration

a larger amount of sugar is burnt off as carbon dioxide in respiration of the yeast.

The increase of concentration increases the property of utilising ammoniacal nitrogen of the yeast and decreases its power of using nitric nitrogen.

The loss of nitrogen is profoundly influenced by the change of concentration in the culture. Thus as the concentration increases from 0.5 per cent. of sucrose to 2.0 per cent. of sucrose in the culture this loss of nitrogen rapidly decreases. This is perhaps due to the reason that the increased oxidation-reaction of the carbohydrate retards the rate of the reduction-reactions which are involved in the loss of nitrogen as nitrogen gas from its compounds.

#### ACKNOWLEDGMENT

I am very much thankful to Prof. N. R. Dhar for his valuable advice in this work.

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# A COMPARATIVE STUDY OF THE GROWTH OF DHAR YEAST AND *SACCHAROMYCES* *CALSBERGENSIS* IN CULTURES, CONTAINING ONLY ETHYL ALCOHOL AS SOURCE OF CARBON UNDER NON-AERATED CONDITIONS

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## INTRODUCTION

It is very well known that several sugars can be utilised as source of carbon food for yeasts. A part of the carbon of these sugars is used up in the formation of yeast cells and a part is oxidised into carbon dioxide and liberates energy needed in the respiration of the cells during their life.

Earlier, Wonogradsky claimed that micro-organisms can also utilise the energy derived from the oxidation of inorganic materials for their growth. This first generalisation of its type was proved correct by Temple and Colmer<sup>1</sup> who could grow *Thiobacillus ferrooxidans* autotrophically by means of the energy derived from the oxidation of ferrous ions. Although such examples, where an organism is found to utilise the energy obtained by ordinary inorganic chemical reactions are rare, it has been seen that many organic compounds can easily be used as source of carbon by micro-organisms. Thus, Starier<sup>2, 3</sup> has shown that ethyl alcohol is oxidised by *Pseudomonas fluorescens* to yield as much as 50 to 70 per cent. of the calculated amount of acetic acid. Alcohol can be oxidised to acetic acid liberating energy needed for the growth of *Streptococcus mastiditis* also, without the accumulation of hydrogen peroxide.<sup>4</sup>

This utilisation of ethyl alcohol as source of carbon is seen also in the case of several yeasts. Thus we see that yeasts are able to live for many years in the liquids which have fermented. It is probable that they use the glycerol and succinic acid which are regarded by Laurent as being able to supply the need of carbon for yeast. But nevertheless for certain species, alcohol seems to be the source of carbon.<sup>5</sup>

Researches of Kayser and Domolon,<sup>6</sup> and Tillat and Aauton,<sup>7</sup> have proved that yeasts oxidise ethyl alcohol to acetaldehyde and this energy is

probably utilised for the growth of yeast. Lindner and Cziser<sup>8</sup> found that ethyl alcohol can be used as source of carbon for yeasts and Stockhausen<sup>9</sup> confirmed this view. Lindner has reported that *Saccharomyces membranæ-faciens* can take its carbon from ethyl alcohol but not from methyl alcohol.<sup>10</sup>

I have seen that under certain conditions both Dhar Yeast,<sup>11</sup> and *Saccharomyces calenbergensis* can grow in cultures containing only ethyl alcohol as source of carbon. In this paper, I am giving a comparative study of the above yeasts in cultures containing other than mineral nutrients, ethyl alcohol as the sole source of carbon, under non-aerated conditions.

#### EXPERIMENTAL

Several cultures as shown in the tables were prepared each containing 0.20 g. of calcium carbonate, 0.25 g. of magnesium carbonate, 0.20 g. of sodium chloride, 0.20 g. of potassium sulphate, 0.20 g. of disodium hydrogen phosphate and 2.5 g. of ammonium sulphate. For this first the minerals were weighed and taken in a 750 c.c. pyrex flat-bottom flask and 200 c.c. of distilled water added to them. These were then digested with dilute hydrochloric acid and the clear solution obtained was cooled and the total volume of the culture made upto 400 c.c. with distilled water, meanwhile adjusting the pH to be 4.5.

The flasks containing the cultures were plugged with surgical cotton and sterilised at a pressure of 10 lb. for 30 minutes in an autoclave. After cooling, the volume of absolute alcohol as given in the tables against each culture was added in each flask and after giving the media a whirling motion, they were seeded with a trace of activated samples of the yeasts.

One flask of every concentration of ethyl alcohol containing all the ingredients was kept as such without seeding to account for the loss of alcohol by evaporation during the period of fermentation. Thus for each concentration of alcohol, three cultures were prepared, one for Dhar Yeast, one for *Saccharomyces calenbergensis* and one for determining the loss of alcohol due to evaporation during fermentation period.

All these cultures were kept together at room temperature, during the period of fermentation. The temperature variation was between 25.5° C. to 29.2° C. The cultures were analysed after 15 days.

#### OBSERVATIONS

The results obtained by the analysis of the above cultures are tabulated below.

The results tabulated are per 100 c.c. of the culture.

TABLE I. *Alcohol Contents*

Ethyl alcohol in the beginning in c.c.	Name of the yeast	Ethyl alcohol present in the culture in the beginning in gm.	Ethyl alcohol lost by evaporation in gm.	Ethyl alcohol left in the culture unconsumed in gm.	Ethyl alcohol consumed in yeast growth in gm.
0.50	Dhar Yeast	0.399	0.249	0.075	0.075
0.50	<i>Saccharomyces calshbergensis</i>	0.399	0.249	0.010	0.140
1.00	Dhar Yeast	0.798	0.268	0.360	0.170
1.00	<i>S. calshbergensis</i>	0.798	0.268	0.310	0.220
2.00	Dhar Yeast	1.596	0.316	0.560	0.720
2.00	<i>S. calshbergensis</i>	1.596	0.316	0.900	0.380
3.00	Dhar Yeast	2.394	1.934	0.790	0.670
3.00	<i>S. calshbergensis</i>	2.394	1.934	0.730	0.730
4.00	Dhar Yeast	3.184	2.054	0.530	0.600
4.00	<i>S. calshbergensis</i>	3.184	2.054	0.430	0.710
5.00	Dhar Yeast	3.980	1.250	1.390	1.340
5.00	<i>S. calshbergensis</i>	3.980	1.250	1.240	1.490
6.00	Dhar Yeast	4.776	2.526	1.500	1.700
6.00	<i>S. calshbergensis</i>	4.776	2.526	1.130	1.120

TABLE II. *Acid Formation*

c.c. of Ethyl alcohol present in the culture in the beginning	Name of the yeast	Total acid produced in gm. equivalents	Volatile acid produced in gm. equivalents	Percentage of acid formation calculated on the basis of alcohol consumed
0.50	Dhar Yeast	0.0675	0.101	90.00
0.50	<i>S. calshbergensis</i>	0.0175	0.005	12.50
1.00	Dhar Yeast	0.0550	0.030	32.35
1.00	<i>S. calshbergensis</i>	0.0350	0.005	29.16
2.00	Dhar Yeast	0.1100	0.020	19.64
2.00	<i>S. calshbergensis</i>	0.0500	0.005	13.16
3.00	Dhar Yeast	0.2750	0.075	41.34
3.00	<i>S. calshbergensis</i>	0.2450	0.130	34.56
4.00	Dhar Yeast	0.2200	0.030	36.66
4.00	<i>S. calshbergensis</i>	0.2700	0.035	38.02
5.00	Dhar Yeast	0.1850	0.055	13.80
5.00	<i>S. calshbergensis</i>	0.2750	0.090	18.45
6.00	Dhar Yeast	0.1900	0.030	11.21
6.00	<i>S. calshbergensis</i>	0.3300	0.145	29.46

TABLE III. Yeast Growth

Ethyl alcohol in the culture in the beginning in c.c.	Name of the yeast	Dry yeast produced in gm.	Percentage of yeast yield calculated on the basis of alcohol consumed
0.50	Dhar Yeast	0.0740	98.66
0.50	<i>S. calenbergensis</i>	0.0760	94.44
1.00	Dhar Yeast	0.0820	48.44
1.00	<i>S. calenbergensis</i>	0.0998	45.32
2.00	Dhar Yeast	0.1920	28.05
2.00	<i>S. calenbergensis</i>	0.1606	17.75
3.00	Dhar Yeast	0.2628	39.20
3.00	<i>S. calenbergensis</i>	0.2586	35.42
4.00	Dhar Yeast	0.3364	56.00
4.00	<i>S. calenbergensis</i>	0.2990	42.11
5.00	Dhar Yeast	0.3310	24.70
5.00	<i>S. calenbergensis</i>	0.2830	19.00
6.00	Dhar Yeast	0.3056	17.90
6.00	<i>S. calenbergensis</i>	0.3300	29.68

## INFERENCE

Consumption of ethyl alcohol by Dhar Yeast is in general less than that of *Saccharomyces calenbergensis*. At lower concentrations of ethyl alcohol higher acid formation is seen in the case of Dhar Yeast, but at higher concentrations, *S. calenbergensis* produces more acid. At all concentrations except at 6 c.c. of ethyl alcohol per 100 c.c. culture, Dhar Yeast grows more than *S. calenbergensis*. This greater consumption of ethyl alcohol followed by less yield of yeast cells in the case of *S. calenbergensis* proves that the rate of respiration of this yeast is faster than that of Dhar Yeast.

## SUMMARY

Dhar Yeast and *S. calenbergensis* can grow in culture containing ethyl alcohol only as source of carbon food under non-aerated conditions. The rate of consumption of alcohol at different concentrations of ethyl alcohol is usually less in the case of Dhar Yeast and is followed by higher yeast yield

as compared to *S. calnsbergensis*. This indicates that the rate of respiration is faster in *S. calnsbergensis*. At low concentration of ethyl alcohol, in the culture, Dhar Yeast produces more acid than *S. calnsbergensis* but at higher concentrations, the latter produces more acid.

#### ACKNOWLEDGEMENT

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# STUDY OF THE MUTATIONS IN DHAR YEAST EFFECTED BY FUCHSIN RED

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## INTRODUCTION

It is well known that certain chemicals when present in the yeast cultures, affect the growth of this organism. Morphological and cytological differences obtained by growing yeasts under different conditions and in cultures containing different nutrients have been studied by Bauch<sup>1, 2</sup> and Subramaniam and Ranganatham,<sup>3</sup> but little work has been done on the physiological differences and the changes in the chemical nature of the yeasts when they are grown in cultures containing any such foreign matter.

I have classified these substances into two groups. To the first type belong those which directly affect the growth of yeast by their presence. This is observed by the analysis of the cultures in which the affecting substances are present.<sup>1, 2</sup> This represents the change of the properties of the yeasts due to the presence of that substance in the culture. The second effect is more deep-rooted. When yeast grows in cultures containing these foreign substances its physiological properties are changed.<sup>4-6</sup> These changes in the properties are specific to the substance added in the culture to effect them. These differences in the physiological properties of the yeast are due to the changes in the internal protoplasmic structure of the yeast,<sup>7</sup> and often can be seen by a good microscope. These substances effect a change in the genes of the cells. If such an affected yeast is taken out from that culture and seeded in a fresh culture it will show physiological properties different from the properties shown by the original yeast grown in another similar culture.

Temporary changes in the physiological properties of yeast are due to the mutation of its genes. It has been established that there is a close relationship between genes and enzymes. These mutated genes produce different enzymes and thus different physiological activities are observed in living tissue. Our knowledge of the relations between genes and enzymes has greatly increased during recent years. American geneticist Beadle has induced mutations of the genes in the spores of *Neurospora* by X-rays and ultra-violet light. Normally this mould can grow in cultures containing

very simple chemicals but after mutation ordinarily unnecessary chemicals must be added in the culture before the mould can grow in it. This loss of the synthetic capacity of the above mould is due to the lack of an enzyme that is present in the normal mould. Numerous such experiments have fully established the close link which exists between genes and enzymes. All the mutations in *Neurospora* do not affect the ability or inability of the formation of a particular enzyme but some mutations affect its form of growth. These mutations have not been fully studied. By studying similar types of mutations in the fruit-fly, *Drosophila*, and the Gypsy Moth, *Lymantria*, it has been found that certain genes show their effect by modifying the rates of the various processes which occur during development and growth.

Certain enzymes depend on the corresponding genes for their formation. Their production is initiated and maintained by the relevant gene. Some enzymes may be formed under the influence of a gene, but once it is formed more will be produced by the cell regardless whether the gene is present or not. If this gene is destroyed and all the enzyme is lost it can be regained. Such enzymes are gene-initiated and not gene-maintained. In my experiments I have induced mutation of yeast genes by chemical agents and have studied the continuity of the changes in the physiological properties of such yeasts.

The mutations in the physiological properties of the yeast thus effected by chemicals are not lasting and by repeatedly growing such mutated yeast in favourable cultures these abnormalities in its physiological properties almost disappear.

These are some dyes which stain these genes without killing the cell. These genes, being complex proteinous matter, are highly specific in nature. A particular dye may stain one or many genes without affecting others.<sup>8</sup> I have tried to change the properties of yeast by growing them in cultures containing fuchsin red dye, which in small amounts does not kill yeast but only stains the cell. This dye affects some genes, by combining with them, which are thus modified in their activities. As mentioned before these affected genes produce changed enzymes which result in changed physiological activities of the yeast.

#### EXPERIMENTAL

Dhar Yeast<sup>9</sup> and a variety of yeast obtained by crossing *Saccharomyces carlsbergensis* with Dhar Yeast were grown in cultures containing fuchsin red. Sucrose was used as source of carbon in the above cultures, which were kept under non-aerated conditions. Each culture contained 0.04 per cent. of the dye.

Activated samples of the above yeasts were prepared from one cell culture, without using the dye. These yeasts were allowed to grow in the above cultures for nearly 25 days and then two drops of the non-dyed and dyed cultures were added in well cotton plugged test-tubes which contained sterilised sucrose cultures. These test tubes-cultures were later on used as seed yeast.

The samples of non-mutated and mutated yeasts separated above were used for seeding the cultures. Several cultures, each containing calcium carbonate 0.2 g., magnesium carbonate 0.25 g., sodium chloride 0.2 g., potassium sulphate 0.2 g., disodium hydrogen phosphate 0.2 g., ammonium sulphate 2.5 g., and sucrose 20 g., were prepared.

First all the above mineral substances were digested in dilute hydrochloric acid and to the clear solution sucrose was added. Total volume of each culture was made up to 400 c.c. and pH adjusted near 4.5. These cultures were kept in 750 c.c. flat-bottom pyrex flasks, cotton-plugged and sterilised by heating for 30 minutes at 15 lb. pressure. After cooling one of the above cultures was seeded with an activated sample of non-mutated Dhar Yeast, second with mutated Dhar Yeast, third with non-mutated Dhar *Saccharomyces carlsbergensis* yeast and fourth with the mutated yeast of this variety.

These cultures were analysed after seven days. Before their analysis a few drops of all the above four cultures were taken out in separate test-tubes containing sterilised cultures for making activated seed yeast.

Four cultures were again prepared as mentioned above and this time the samples of yeast obtained from the above cultures were used for seeding. This time also two of the flasks were seeded with non-mutated samples of the abovementioned two yeasts and two with the mutated varieties. These cultures were analysed after 28 days. A few drops of each of these cultures were again taken out in test-tubes containing normal sterilised cultures, to prepare activated seed yeast for further experiments, before this analysis.

Four cultures, each containing all the substances as described above, were again prepared and they were seeded with the above two non-mutated and two mutated samples of the yeasts. These cultures were analysed after 24 days.

#### OBSERVATIONS

*First Analysis.*—The results obtained by the analysis of the above cultures are tabulated below:—First analysis: Study of the effect of fuchsin red dye on yeast cultures using 5 per cent. (W/v) of sucrose (20 g. in 400 c.c.)

as source of carbon food, under non-aerated conditions. All the cultures were kept at 30° C.

TABLE I. Table showing the data concerning Sugar Fermentation

Sl. No.	Name of the yeast used for seeding	Period of fermentation in days	g. of fuchsin red added in the culture	g. of sucrose in the culture in the beginning	g. of sugar left in the culture	g. of sugar consumed during fermentation	Percentage of sugar fermented
1	Dhar Yeast	.. 26	Nil	20.00	2.73	17.27	86.35
2	Dhar Yeast	.. 26	0.05	20.00	6.32	13.68	68.40
3	Dhar Yeast <i>Saccharomyces carlsbergensis</i>	30	Nil	20.00	1.54	18.46	92.30
4	Dhar Yeast <i>Saccharomyces carlsbergensis</i>	30	0.05	20.00	2.73	17.27	86.35

TABLE II. Table indicating Acid Formation

S. No. according to Table I	g. equi. of total acid produced	g. equi. of volatile acid formed	g. equi. of non-volatile acid formed	Percentage of acid formation calculated on the basis of sugar consumed during fermentation
1	1.7720	0.0381	1.7339	10.00
2	1.7972	0.0286	1.7686	13.20
3	1.7720	0.0095	1.7625	9.69
4	1.7120	0.0382	1.7138	10.00

TABLE III. Table showing Alcohol Formation and Yeast Growth

Sl. No. according to Table I	g. of ethyl alcohol formed during fermentation	Percentage of alcohol formation calculated on the basis of sugar consumed	g. of yeast grown in the culture during fermentation	Percentage of yeast yield calculated on the basis of sugar consumed
1	1.24	7.18	3.4412	19.34
2	0.24	1.75	3.5650	26.09
3	1.24	6.71	3.0066	16.29
4	1.24	7.18	3.4412	19.34

*Second Analysis.*—Before the analysis of the cultures of the first set a few drops of each culture were taken out in sterilised cultures contained in test-tubes. After activating these yeasts they were used as seeds in the following experiments. In the beginning each culture contained 10 g. of sucrose per 200 c.c. During the period of fermentation temperature was maintained at 25° C. and cultures were analysed after 15 days.

TABLE IV. *Table showing Fermentation Data*

S. No.	Name of the yeast in culture	Nature of the yeast	g. of reducing sugar left in the culture	g. of non-reducing sugar left	g. of total sugar left	g. of sugar consumed during fermentation	% of sugar consumed cal. on the basis of sugar originally present
1	Dhar Yeast	.. Normal	5.00	2.19	7.19	2.81	28.10
2	Dhar Yeast	.. Mutated	5.26	2.43	7.69	2.31	23.10
3	Dhar Yeast <i>Saccharomyces carlsbergensis</i>	Normal	5.15	1.43	6.58	3.42	34.20
4	Dhar Yeast <i>Saccharomyces carlsbergensis</i>	Mutated	5.58	1.08	6.66	3.34	33.40

TABLE V. *Table showing Acid Formation, Alcohol Produced and Yeast Growth*

S. No. according to Table IV	g. equi. of total acid produced during fermentation	g. equi. of volatile acid formed	g. equi. of non-volatile acid formed	Percentage of acid formation calculated on the basis of sugar consumed	g. of ethyl alcohol formed during fermentation	Percentage of alcohol formation cal. on the basis of sugar consumed	g. of dry yeast grown during fermentation	Percentage of yeast yield cal. on the basis of sugar consumed
1	0.7708	0.0141	0.7561	27.40	0.22	7.82	0.9372	33.45
2	0.8648	0.0141	0.8507	37.23	0.00	0.00	1.5656	67.95
3	0.7896	0.0094	0.7802	23.09	0.06	1.75	0.9680	28.39
4	0.7520	0.0005	0.7515	22.45	0.00	0.00	1.4037	43.71

*Third Analysis.*—Study of the residual effect of mutation induced by fuchsin red dye in the yeasts after they had been grown in ordinary sucrose cultures for 15 days:—Traces of samples of yeast obtained from the culture of the second set, before analysis, were added in test-tubes containing sterilised cultures. After due activation these yeasts were used as seed in the following experiments. Each culture contained 10 g. of sucrose in the beginning in 200 c.c. of the culture. The temperature during fermentation was 20° C. and the cultures were analysed after 30 days.

TABLE VI. Table showing Sugar Fermentation Data

S. No.	Name of the yeast used in the culture	Nature of the yeast	g. of total sugar left in the culture after fermentation	g. of reducing sugar left after fermentation	g. of non-reducing sugar left	g. of sugar consumed during fermentation	% of sugar consumed cal. on the basis of original sugar
1	Dhar Yeast ..	Normal	5.03	4.58	0.45	4.97	49.70
2	Dhar Yeast ..	Mutated	2.91	2.35	0.56	7.09	70.90
3	Dhar Yeast <i>Saccharomyces carlsbergensis</i>	Normal	3.87	3.76	0.11	6.13	98.90
4	Dhar Yeast <i>Saccharomyces carlsbergensis</i>	Mutated	4.16	3.61	0.55	5.84	94.50

TABLE VII. Table showing Acid Formation, Alcohol Production and Yeast Yield

S. No. according to Table VI	g. equi. of total acid formed during fermentation	g. equi. of volatile acid produced	g. equi. of non-volatile acid formed	% of acid formation cal. on the basis of sugar consumed	g. of alcohol formed during fermentation	% of alcohol formation cal. on the basis of sugar consumed	g. of dry yeast grown in the culture during fermentation	% of yeast yield cal. on the basis of sugar consumed
1	0.8270	0.0094	0.8178	16.50	0.64	6.90	1.1941	24.02
2	1.2220	0.0094	1.2126	10.60	0.20	2.82	1.8364	25.90
3	0.8836	0.0328	0.8508	14.34	0.84	13.70	1.5490	25.46
4	0.8460	0.0282	0.8178	14.55	0.10	1.71	1.2538	20.79

*Fourth Analysis.*—Study of the residual effect of mutation, induced by fuchsin red dye in the yeasts, after they had been grown in ordinary sucrose cultures for nearly two months:—Before the analysis of the third set a few drops of each culture were taken out in separate test-tubes containing sterilised sucrose cultures. After proper activation of these samples they were used for seeding in the following experiments. Each culture of this set contained 10 g. of sucrose in 200 c.c. They were kept, like other sets, under non-aerated conditions at 20° C. The period of fermentation was 30 days.

TABLE VIII. Table showing Sugar Consumption by the Yeasts

S. No.	Name of yeast used in fermentation	Nature of the yeast	g. of total sugar left after fermentation	g. of reducing sugar left	g. of non-reducing sugar left	g. of sugar consumed during fermentation	Percentage of sugar consumed calculated on the basis of sugar used up
1	Dhar Yeast ..	Normal	6.06	5.81	0.25	3.94	39.40
2	Dhar Yeast ..	Mutated	6.89	6.89	0.00	3.11	31.10
3	Dhar Yeast <i>Saccharomyces carlsbergensis</i>	Normal	5.92	5.92	0.00	4.08	40.80
4	Dhar Yeast <i>Saccharomyces carlsbergensis</i>	Mutated	6.02	5.55	0.47	3.98	39.80

TABLE IX. Table showing Acid Formation, Alcohol Formation and Yeast Yield

S. No. according to Table VIII	g. equi. of total acid formed	g. equi. of volatile acid formed	g. equi. of non-volatile acid formed	Percentage of acid formation cal. on the basis of sugar consumed	g. of ethyl alcohol produced during fermentation	Percentage of alcohol formation cal. on the basis of sugar consumed	g. of dry yeast grown during fermentation	Percentage of yeast yield calculated on the basis of sugar consumed
1	0.4112	0.0705	0.3407	8.63	0.20	5.03	1.1762	29.84
2	0.4112	0.0235	0.3877	12.21	0.10	3.20	1.2202	39.23
3	0.4528	0.0705	0.3823	11.02	0.42	10.29	1.1864	29.07
4	0.4112	0.0235	0.3877	9.80	0.20	5.03	1.0452	26.26

## DISCUSSION

When Dhar Yeast is grown in cultures containing  $1.25 \times 10^{-3}$  per cent. of fuchsin red dye the acid-forming and yeast-growing properties of the yeast are increased but its sugar-consuming and alcohol-forming properties show a decrease. The yeast obtained by crossing Dhar Yeast with *Saccharomyces carlsbergensis* has a strong fuchsin-resisting property. The fermenting power of this yeast is decreased and acid-forming, alcohol-producing and yeast-growing properties are increased in fuchsin cultures although these differences are much less as compared to the differences effected by growing Dhar Yeast in similarly fuchsinated culture.

When these mutated yeasts are used as seed-yeasts in the similarly prepared sucrose cultures it is observed that the changes in these yeasts have so affected that those differences which are noticed in the first set of cultures containing fuchsin dye, continue even afterwards without the presence of fuchsin in the latter cultures. This proves that the changes in the analytical values obtained by the analysis of the fuchsinated and non-fuchsinated cultures were due to the changes in the physiological nature of the yeasts effected by chemical changes brought about in the genes of the yeasts by fuchsin.

Dhar Yeast, being greatly affected by fuchsin red, shows these differences in a more marked manner as compared to the other variety which is feebly attacked by this chemical. The nature of these persisting physiological difference is almost the same as that observed in the fuchsinated cultures. Thus Dhar Yeast which shows a slight increase of yeast yield in fuchsin cultures, shows a good increase of yeast yield in the cultures of the second set which were analysed after 15 days. The difference of the two values is more than what was observed in fuchsinated and non-fuchsinated (normal)

culture because the fuchsinated cultures contained fuchsin red dye which had definite poisonous effect on yeast growth. A similar observation has been made in the case of the other variety of yeast. These changes are more marked when the cultures are analysed just after a few days of seeding and appear less evident as the cultures become old.

These differences continued for nearly three months and gradually diminished. This gradual returning to the normal, after several uncared generations, is also seen in almost all artificially cultivated varieties even if cross-fertilisation might have been applied to obtain the requisite change.

#### SUMMARY

The changes in the physiological properties of yeasts can be induced by growing them in cultures containing fuchsin red, for nearly a month. This dye does not kill the yeast but affects the protoplasmic contents of the cells. The genes of the cells are affected, these affected genes produce changed enzymes which are responsible for the differences in the physiological properties of the yeast. This is observed that these changes slowly disappear by growing these mutated yeasts in normal cultures for several months.

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# A STUDY OF THE INFLUENCE OF HYDROGEN-ION CONCENTRATION ON THE SYNTHESIS OF AMINO ACIDS FROM PARAFORMALDEHYDE AND POTASSIUM NITRATE

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THE role of light in the synthesis of amino acid is still not fully established.<sup>1</sup> There are some evidences that in plants proteins are synthesised in leaves where there is a sufficient supply of carbohydrate and that too more in light than in dark.<sup>2</sup> The nitrogen of these proteins comes from nitrites. Shimper<sup>3</sup> has observed that nitrites are always present in the living leaf in the dark and disappears in the light. Probably in the presence of light this nitrite nitrogen gets reduced to amino nitrogen and in this form it joins the carbon chain and gives rise to amino acids which are later converted into proteins.

We have been successful in synthesising amino acids from a suspension of paraformaldehyde and potassium nitrate in water containing a little of ferric chloride as catalyst. When this mixture is exposed to sunlight for about 80 hours, about a dozen of amino acids are synthesised in it.<sup>6</sup>

Here in this paper we have studied the influence of hydrogen-ion concentration on this synthesis using an aqueous solution of citric acid and disodium hydrogen phosphate solution mixture as the buffer medium.

## EXPERIMENTAL

In five separate 250 c.c. Pyrex beakers 2 g. of paraformaldehyde, 0.55 g. of potassium nitrate and 1 c.c. of 6 N. ferric chloride solution were taken. To these beakers were added calculated amounts of citric acid and disodium hydrogen phosphate to give pH values, 2.2, 3, 4, 5 and 6 respectively when 100 c.c. of distilled water were added. 100 c.c. of distilled water were poured in each beaker and they were covered with Pyrex beaker covers and exposed to sunlight. The temperature variation during the period of exposure was between 16° and 25° C.

The formation of amino acids in these suspensions was detected by Circular Paper Chromatography<sup>4, 5</sup> using butanol-acetic-acid-water mixture

as the solvent and triketohydrindene hydrate (ninhydrin) as the colour producing reagent.

## OBSERVATION

(1) The mixture at pH 2.2 gave the following results:—

Hours of exposure	Amino acids detected
50	.. Lysine
100	.. Leucine, Isoleucine Valine, Histidine and Lysine
150	.. Lysine, Valine, Histidine and Proline
200	.. Lysine, Valine, Histidine and Proline

The pH of the mixture changed to 2.3 at the end of 200 hours exposure.

(2) The mixture at pH 3 gave the following results:—

Hours of exposure	Amino acids formed
50	.. Lysine
100	.. Lysine, Valine, Histidine and Proline
150	.. Lysine, Valine, Histidine, Proline, Asperagine, Glutamic acid and Serine
200	.. Lysine, Valine, Histidine, Proline, Asparatic acid, Glutamic acid and Serine

The pH of the mixture changed to 3.9 at the end of 200 hours exposure.

(3) The mixture at pH 4 gave the following data:—

Hours of exposure	Amino acids formed
50	.. Lysine
100	.. Lysine, Valine, Histidine, Proline and Serine
150	.. Lysine, Valine, Histidine, Proline, Serine, Arginine and Glycine
200	.. Lysine, Valine, Histidine, Proline, Serine, Arginine and Glycine

The pH of the mixture changed to 6.4 after 200 hours of exposure,

(4) The mixture at pH 5 gave the following results:—

Hours of exposure	Amino acids formed
50	.. Glutamic acid
100	.. Valine, Histidine, Serine, Arginine and Proline
150	.. Valine, Histidine, Proline, Alanine, Threonine, Glycine, Arginine, Serine and Glutamic acid
200	.. Proline, Glycine

The pH of the mixture changed to 6·8 after 200 hours of exposure.

(5) The mixture at pH 6 gave the following results:—

Hours of exposure	Amino acids formed
50	.. Glutamic acid
100	.. Leucine, Lysine, Histidine, Serine and Valine
150	.. Valine, Histidine, Lysine, Serine, Arginine and Glycine
200	.. Valine, Histidine, Proline and Glycine

#### DISCUSSIONS

In the mixtures at pH 2·2, 3 and 4 lysine is synthesised first but in the mixtures at pH 5 and 6 glutamic acid is first formed. Leucine is formed only at pH 2·2 and 6 and it disappears on long exposure. Valine, histidine and proline are synthesised in the mixtures at all pH studied. Serine appears in cultures of pH 3, 4, 5 and 6 only.

It is interesting that though the pH of a mixture containing no buffer decreases on exposure to sunlight as shown in my previous papers published elsewhere<sup>6</sup> the pH of the cultures, containing citric acid and disodium hydrogen phosphate as buffer, increases. Thus the mixtures at pH 2·2, 3, 4, 5 and 6 have pH 2·3, 3·9, 6·4, 6·8 and 7·1 after 200 hours of exposure. The only explanation which can be given at this stage is that probably citric acid is converted to some other non-acidic or less acidic compound during this exposure and this leaves excess disodium hydrogen phosphate which makes the mixture alkaline,

Some of the amino acids formed during exposure do not remain in the mixture for long. They are either used up in the formation of some other amino acids which appear afterwards or they combine with other amino acids and form peptides.

#### SUMMARY

When a mixture of paraformaldehyde, potassium nitrate, water, a little ferric chloride as catalyst and citric acid and disodium hydrogen phosphate as buffer is exposed to sunlight several amino acids are formed in it. The mixtures at different pH show the synthesis of different amino acids. The mixtures at pH 2.2, 3 and 4 indicate the formation of lysine first but those at pH 5 and 6 show the formation of glutamic acid to start with.

On prolonged exposure each mixture shows the synthesis of several other amino acids many of which remain in the mixture even after long exposure, but a few of them disappear in the course of exposure.

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# CARBON-HALOGEN BOND ENERGY IN MONO-HALIDES OF NORMAL PARAFFINS

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(Communicated by Dr. K. Majumdar)

## ABSTRACT

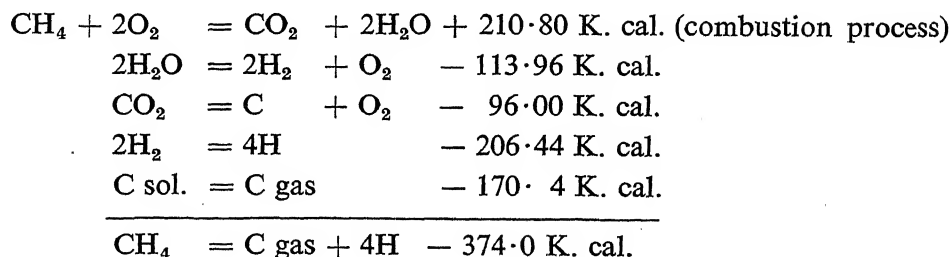
Average carbon-hydrogen and carbon-carbon bond energies in normal paraffins have been calculated from thermochemical and spectroscopic data. With their help the carbon-halogen bond energies in mono-halogen derivatives of normal hydrocarbons have been calculated.

## INTRODUCTION

SEN GUPTA (1953) in a recent communication has calculated the carbon-halogen bond energies in the halogen substituted derivatives of methane. Here an attempt has been made to extend the method to calculate the carbon-halogen bond energies in the monohalides of higher paraffins, with the fresh value of the dissociation energies.

## CALCULATION OF BOND ENERGIES

Calculation of the carbon-halogen bond energy requires a knowledge of average carbon-hydrogen and carbon-carbon bond energies. For methane the atomic heat of formation is given by



So the average C—H bond energy = 93.5 K. cal. To calculate C—C bond energy let us for the instant assume that in the ethane the average C—H bond energy remains same. From combustion data of ethane its heat of formation is calculated to be 641 K. cal. So the C—C bond energy is given by  $(641 - 6 \times 93.5)$  K. cal. = 80 K. cal. Now combustion data for propane gives its atomic heat of formation equal to 907.7 K. cal. Therefore, the average



TABLE II  
*C—X Bond Energies*

Spectroscopic state of C	$s^2 p^2$	3P	$s^2 p^2$	ID	$s^2 p^2$	I	Cl	Br	$s^2 p^2$	IS	$s^2 p^3$	5S	I
Paraffin ..	..	Br	I	Cl	Br	I	Cl	Br	I	Cl	Br	I	
Methane ..	88.78	62.92	41.47	117.78	91.92	70.47	150.28	124.42	102.97	184.78	158.92	137.47	
Ethane ..	93.64	64.52	42.25	122.64	93.52	71.25	155.14	126.02	103.75	189.64	160.52	138.25	
Propane ..	89.94	65.42	43.17	118.94	94.42	72.17	151.44	126.92	104.67	185.94	161.42	139.17	
Isobutane ..	90.49	..	..	119.49	..	..	151.99	..	..	186.49	..	..	

C—C bond energy is given by  $\frac{1}{2}(907.7 - 8 \times 93.5) = 79.85$  K. cal. which is in very good agreement with the value calculated from ethane. This proves the validity of our assumption, namely, the average bond energy is same throughout the homologous series.

The carbon-halogen bond energies in monohalides of ethane have been calculated in Table I. Similar calculations have also been made for propane and isobutane.

#### DISCUSSION

From the table we notice that the value of the carbon-halogen bond energy is fairly constant for the homologous series, with a slight increasing

TABLE III  
*Data used in Calculations*

Heat of Combustion:	K. cal.	
CH <sub>4</sub>	210.8	
CH <sub>3</sub> Cl	164.0	
CH <sub>3</sub> Br	184.0	
CH <sub>3</sub> I	200.5	
C <sub>2</sub> H <sub>6</sub>	368.4	
C <sub>2</sub> H <sub>5</sub> Cl	316.7	
C <sub>2</sub> H <sub>5</sub> Br	340.0	
C <sub>2</sub> H <sub>5</sub> I	358.0	
C <sub>3</sub> H <sub>8</sub>	526.3	
C <sub>3</sub> H <sub>7</sub> Cl	478.3	
C <sub>3</sub> H <sub>7</sub> Br	497.0	
C <sub>3</sub> H <sub>7</sub> I	514.3	
C <sub>4</sub> H <sub>10</sub> (Isobutane)	683.4	
C <sub>4</sub> H <sub>9</sub> Cl	635.5	
Heat of formation:		
CO <sub>2</sub>	94	
H <sub>2</sub> O	56.98	
Heat of dissociation:		
Cl <sub>2</sub>	57.8	
Br <sub>2</sub>	45.44	
I <sub>2</sub>	35.55	
H <sub>2</sub>	103.22	
Spectroscopic term differences for C atom:		
s <sup>2</sup> p <sup>2</sup> 3P	s <sup>2</sup> p <sup>2</sup> 1D	29.0
s <sup>2</sup> p <sup>2</sup> 3P	s <sup>2</sup> p <sup>2</sup> 1S	61.5
s <sup>2</sup> p <sup>2</sup> 3P	sp <sup>3</sup> 5S	96.0

tendency, excepting in ethyl chloride. As we pass from chlorine to iodine the C—X bond energy gradually decreases showing thereby their increasing unstability. This explains the occurrence of CF<sub>2</sub>, CCl, CBr and no CI bands in the excitation of halogen derivatives of normal hydrocarbons.

In the above calculations the generally accepted value for the heat of sublimation of carbon has been used. Gaydon and Penney's (1945) value for the dissociation energy of CO determined by non-crossing rule and Brewer-Gilles-Jenkins' (1948) vapour pressure determination of graphite suggests a value round-about 170·4 K. cal., which is mostly accepted. Table III gives the data used in calculation.

The thermochemical data have been taken from *International Critical Tables* (1928) and the spectroscopic data from Gaydon (1947).

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# THE AZIMUTHAL EFFECT OF COSMIC RAYS AT GULMARG

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## ABSTRACT

Directional variation of hard component of cosmic rays was measured at Gulmarg (8,890 ft.,  $24^{\circ} 36'$  N. geomagnetic latitude). The telescopes were  $180^{\circ}$  to each other in the horizontal plane. At fixed zenith angles of  $40^{\circ}$  and  $60^{\circ}$  the azimuths were changed by steps of  $15^{\circ}$ . Curves so obtained were found to be in agreement with theoretical curves.

MEASUREMENTS on the azimuthal effect at intermediate latitudes have been reported by Gill,<sup>1</sup> Vallarta,<sup>1</sup> Gill and Vaze.<sup>3</sup> These measurements help to determine the energy spectrum of primary cosmic rays.

With a view to study the azimuthal effect at mountain altitudes in the intermediate latitudes, the present experiment was performed at the Gulmarg Research Observatory, Gulmarg, Kashmir, at 8,890 ft.,  $24^{\circ} 36'$  N. geomagnetic latitude. The observations cover the period from August 3 to November 2, 1952. The apparatus consisted of two triple coincidence cosmic-ray telescopes, having three trays of externally coated counters. Each tray had five counters joined in parallel covering an area of 100 sq. cm. The cone covered by each telescope was  $23^{\circ}$  by  $17^{\circ}$ . 12 cm. of lead was inserted in each telescope to filter out the soft component of cosmic rays. A, B and C trays (Fig. 1) were in coincidence, while side counters D were in anti-coincidence to minimise the effect of the side showers. Recording of counts was made on a paper attached to a moving drum. Both the telescopes were mounted on a wooden board. The board was rotated in the horizontal plane in steps of  $15^{\circ}$  through an angle of  $360^{\circ}$ . Telescopes were directed at a common zenith angle of either  $0^{\circ}$ ,  $40^{\circ}$  or  $60^{\circ}$ . The apparatus was placed in a special tower-like room having wooden wall on all sides. The temperature of the room was kept within a range of a few degrees. The data are shown in Tables I, II, and III.

TABLE II

Zenith angle 60°. Telescope (1) Curve (i)

Azimuthal angle	Counts per minute	Error	Azimuthal angle	Counts per minute	Error
0	·948	·025	195	·999	·027
15	·966	·023	210	·981	·029
30	·933	·028	225	·943	·030
45	·893	·058	240	·972	·024
60	·899	·031	255	1·002	·025
75	·860	·026	270	1·004	·021
90	·843	·026	285	1·030	·025
105	·829	·025	300	1·0315	·025
120	·898	·023	315	·987	·026
135	·909	·026	330	·935	·027
150	·873	·099	345	·999	·029
165	·939	·028	360	·948	·026
180	·942	·027			

TABLE III

Zenith angle 60°. Telescope (2) Curve (ii)

Azimuthal angle	Counts per minute	Error	Azimuthal angle	Counts per minute	Error
0	·906	·024			
15	·918	·022	195	·850	·026
30	·954	·026	210	·926	·027
45	·837	·025	225	·887	·030
60	·822	·029	240	·917	·025
75	·822	·024	255	·887	·025
90	·824	·024	270	·922	·029
105	·798	·027	285	·980	·026
120	·844	·047	300	·936	·026
135	·862	·047	315	·947	·027
150	·807	·052	330	·909	·038
165	·806	·052	345	·919	·029
180	·872	·052	360		

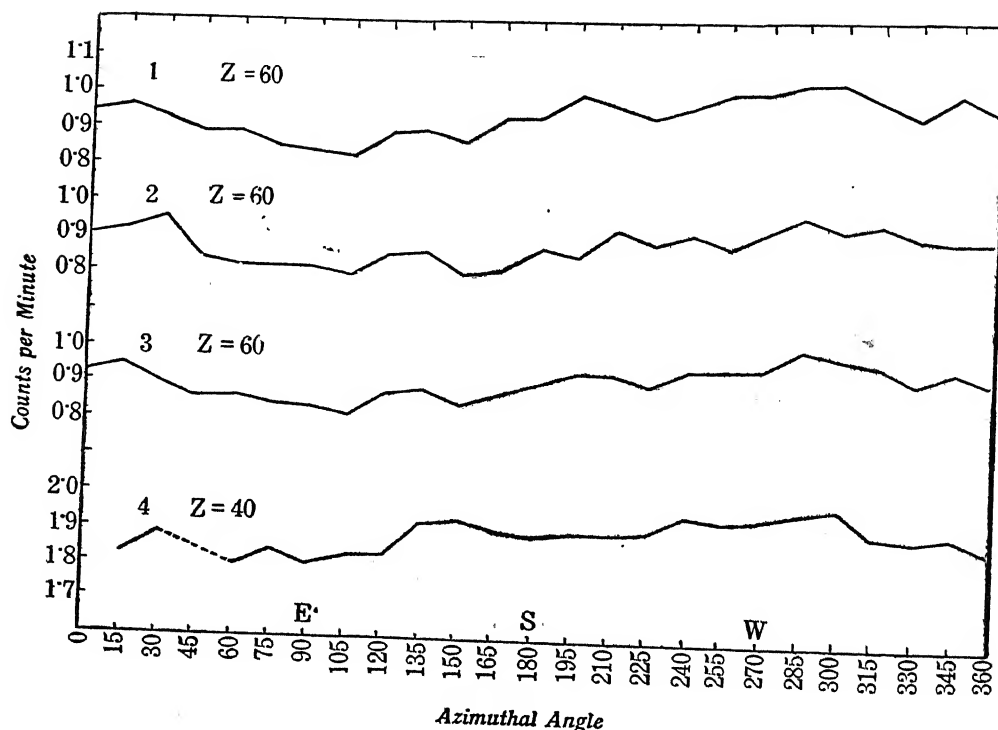


FIG. 2

Curves (1), (2) represent the number of counts per minute obtained separately by the two telescopes at steps of  $15^\circ$  along the azimuths for a zenith angle of  $60^\circ$ . Curve (3) represents the average number of counts per minute of both the telescopes. Curve (4) represents the number of counts per minute for zenith angle of  $40^\circ$ . All the curves show irregularities in the north-western quadrant, and the  $60^\circ$  zenith curves (1) and (2) are similar.

The irregularities of the curves are in agreement with theoretical curves of Hutner<sup>4</sup> and experimental curves of Gill. Gill's<sup>6-10</sup> curves also have prominent irregularities in the N.-W. quadrant. According to Alpher's<sup>11</sup> theoretical curves the maximum of the curves at  $25^\circ$  N. geomagnetic latitude should occur near about the azimuth of  $280^\circ$ . In 1945 Gill reported that at  $22^\circ$  N. the maximum azimuthal variation for hard component penetrating 10.2 cm. of lead does not occur for the east-west magnetic meridian but near about  $280^\circ$ . East-west asymmetry according to Table II for the zenith angle of  $60^\circ$  is 14.4%, and for  $40^\circ$  zenith angle it is only about 4%. Vallarta *et al.*<sup>5</sup> observed the asymmetry of the penetrating component of cosmic rays at Mexico City ( $29^\circ$  geomagnetic latitude) about 14% for zenith angle of  $60^\circ$  and about 9% for zenith angle of  $40^\circ$ .

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# THE COMPOSITION AND QUALITY OF MAIZE GRAINS AS AFFECTED BY VARIETIES, MANURES AND SPACINGS

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AND

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*(Communicated by Prof. S. Ghosh)*

INVESTIGATIONS on the composition and quality of food grains as affected by varietal, manurial or other cultural practices in India are extremely meagre. The question of quality in crops in relation to cultural practices has been tackled for some of the cash crops, like sugarcane, cotton, tea, etc.; but for ordinary food crops such studies are comparatively few. Mukerji and Agarwal (1944) made a preliminary study of the influence of variety, manures and irrigation on the composition and quality of Indian barley for malting or feeding purposes.

Some work on the chemical composition of maize grains as affected by varietal, manurial or other cultural differences has been reported from the U.S.A. According to Hetzger (1933) there is a gradual increase in the crude fibre contents of mature kernels of maize as the ratio between nitrogen and phosphorus in the fertilizers becomes wider. In a study on the composition of maize grown under field conditions in relation to the soil and its treatments, Weeks, Fergus and Karraker (1941) found that nitrogenous treatments to soil gave as high a protein in grains as in soils where no nitrogen was added. Fat and fibre contents also did not change consistently by soil treatments. However, the results of the experiments conducted at the Indiana Agricultural Experiment Station (1945) show that the protein content of maize grains varied from 7.8 per cent. on poor silt loam soils receiving only phosphorus and potash to 9.4 per cent. on soils where 1,000 lb. of sulphate of ammonia per acre was added in addition to phosphorus and potash. In later studies reported by Weeks and Fergus (1946) and Weeks and Walters (1947) it is stated that the composition of maize grains seems to be as variable as yield and was influenced by environmental factors. These authors emphasise the effect of phosphorus on grain composition and report that of all soil treatments phosphorus fertilization had the greatest effect

on composition specially in regard to the phosphorus content of grains which varied from 12 per cent. in control plots to 55 per cent. in plots consistently treated with phosphatic fertilizers.

From the above review it is evident that fertilizers and other soil treatments considerably affect the chemical composition of maize grains. In order to elucidate some of these points under Indian conditions a systematic study on the composition of maize grains as affected by varieties, manures and spacings was undertaken at Kanpur.

#### EXPERIMENTAL

Samples of maize grains from a complex varieties *cum*-nitrogen *cum*-spacing trial conducted by the Economic Botanist (Oilseeds) to Government, U.P., Kanpur, during 1949-50 at the Government Research Farm, Kalyanpur (near Kanpur), were obtained plot-wise for analysis. The grain samples were secured from the bulk produce of each of the 36 treatment combination plots in a manner so that a representative sample is obtained for the laboratory tests. The sample so received was examined in the laboratory for 1,000 corn weight, crude proteins, crude fats, crude fibre and total minerals. The total carbohydrates in the sample were obtained by difference in the usual manner. The methods recommended for feeds and feeding stuff in the A.O.A.C. were adopted for the estimations of the above constituents. Detailed results of analysis are given in Table I.

In the trial there were three varieties, *viz.*, T 41, U.S.A. hybrid and T 4111. T 41 is a local selection of maize from the light-yellow bold seeded Jaunpur varieties and T 4111 an early maturing yellow medium seeded selection from Aligarh. These two are the usual improved varieties of maize recommended by the Department of Agriculture, U.P., for distribution to cultivators. They were, therefore, compared with an U.S.A. hybrid in respect of their relative performances. Besides the varietal differences, three levels of nitrogen were tested, *viz.*, no nitrogen, 40 lb. nitrogen and 80 lb. nitrogen per acre in the form of ammonium sulphate over a basal dressing of 40 lb. nitrogen as farmyard manure in the trial. Four spacings, *i.e.*,  $2' \times 1'$ ,  $2' \times 2'$ ,  $3' \times 1'$  and  $3' \times 2'$  (figures represent distances from row to row and plant to plant respectively) were also included in the treatments to study the relation of plant population on varietal performances as affected by nitrogenous fertilization. These spacings give a plant population of roughly 21,800; 10,900; 14,500 and 7,250 plants per acre respectively in the treatments mentioned. The soil where the trial was conducted is a loam classified by the U.P. Soil Survey Organisation as Kanpur Type 2 (Agarwal

TABLE I  
Detailed results of Analysis of Maize Grains-variatal-cum-nitrogen-cum-spacing trial (1949-50)  
(Oven dry basis)

Particulars	N <sub>0</sub>			N <sub>1</sub>			N <sub>2</sub>		
	S <sub>0</sub>	S <sub>1</sub>	S <sub>2</sub>	S <sub>3</sub>	S <sub>0</sub>	S <sub>1</sub>	S <sub>2</sub>	S <sub>3</sub>	S <sub>3</sub>
Weight of 1,000 grains (gm.)	159	176	158	194	172	201	190	184	206
Crude proteins %	9.68	9.71	10.23	10.89	8.06	9.76	10.03	9.63	10.85
Crude fat %	4.18	4.31	4.72	4.50	4.62	4.42	4.80	4.83	4.69
Crude fibre %	2.10	1.77	1.71	2.69	1.70	1.73	2.73	2.74	1.78
Mineral matter %	1.53	1.63	1.58	1.57	1.46	1.51	1.51	1.60	1.60
Carbohydrates %	82.5	82.6	81.8	80.4	84.2	82.6	80.9	81.2	81.1
Variety—Kanpur T 41									
Weight of 1,000 grains (gm.)	179	192	166	180	165	184	181	182	183
Crude proteins %	10.69	10.90	10.94	9.79	10.46	9.35	10.30	9.65	9.84
Crude fat %	3.81	3.65	3.60	3.66	3.77	3.73	3.79	3.31	3.60
Crude fibre %	2.02	1.93	1.94	1.89	2.01	2.02	1.93	1.89	1.95
Mineral matter %	1.73	1.71	1.85	1.66	1.73	1.75	1.64	1.73	1.74
Carbohydrates %	81.8	81.8	81.7	83.0	82.0	83.2	82.4	83.4	82.9
Variety—U.S.A. hybrid									
Weight of 1,000 grains (gm.)	142	146	138	144	130	149	161	149	169
Crude proteins %	10.62	10.52	10.15	11.17	9.87	10.19	11.67	10.29	10.56
Crude fat %	4.62	4.40	4.68	4.90	4.65	4.63	4.43	4.70	4.81
Crude fibre %	1.72	1.59	1.39	1.76	1.74	1.49	1.52	1.91	1.77
Mineral matter %	1.60	1.53	1.70	1.64	1.59	1.59	1.62	1.63	1.53
Carbohydrates %	81.4	82.0	82.1	80.5	82.2	82.1	80.8	81.5	81.3
Variety—Kanpur T 4111									
Weight of 1,000 grains (gm.)	179	192	166	180	165	184	181	182	183
Crude proteins %	10.69	10.90	10.94	9.79	10.46	9.35	10.30	9.65	9.84
Crude fat %	3.81	3.65	3.60	3.66	3.77	3.73	3.79	3.31	3.60
Crude fibre %	2.02	1.93	1.94	1.89	2.01	2.02	1.93	1.89	1.95
Mineral matter %	1.73	1.71	1.85	1.66	1.73	1.75	1.64	1.73	1.74
Carbohydrates %	81.8	81.8	81.7	83.0	82.0	83.2	82.4	83.4	82.9
Variety—U.S.A. hybrid									
Weight of 1,000 grains (gm.)	142	146	138	144	130	149	161	149	169
Crude proteins %	10.62	10.52	10.15	11.17	9.87	10.19	11.67	10.29	10.56
Crude fat %	4.62	4.40	4.68	4.90	4.65	4.63	4.43	4.70	4.81
Crude fibre %	1.72	1.59	1.39	1.76	1.74	1.49	1.52	1.91	1.77
Mineral matter %	1.60	1.53	1.70	1.64	1.59	1.59	1.62	1.63	1.53
Carbohydrates %	81.4	82.0	82.1	80.5	82.2	82.1	80.8	81.5	81.3

Over a basal dose of 40 lb. N per acre as farmyard manure

$N_0$  = No nitrogen  
 $N_1$  = 40 lb. N per acre  
 $N_2$  = 80 lb. N per acre

$S_0$  = 2' x 1' spacing  
 $S_1$  = 2' x 2' spacing  
 $S_2$  = 3' x 1' spacing  
 $S_3$  = 3' x 2' spacing

and Mehrotra, 1952). The soil is slightly mature of a dark gray colour, non-calcareous and with a pH value of about 7.8. It rests on a subsoil which is darker in colour and more clayey in texture. It is poor in nitrogen and medium in phosphorus contents.

A description of the relative yields of maize grains obtained through the various treatments is of interest in assessing the value of chemical composition of grains since it is ultimately the yield per acre which decides the superiority or otherwise of a particular treatment. It may be mentioned that highest yield per acre was obtained through the variety T 41 although there was no significant difference between the yield of this variety and that of T 4111. The yield of the U.S.A. hybrid was significantly low. In regard to the nitrogen doses, the yields increased progressively with an increase in the amount of nitrogen fertilizer added as ammonium sulphate. Maximum yield was secured through 80 lb. nitrogen supplemented over the 40 lb. basal dressing. Decreasing the plant population decreased the yield, maximum yield having been obtained with a spacing of 2' × 1' in the trial.

The mean values for various constituents obtained in the course of chemical analysis of grains for the various treatments are given in Table II

TABLE II  
*Mean Values for the Chemical Analysis of Maize Grains*  
(Oven-dry basis)

Treatment	Weight 1,000 grains (gm.)	Crude Proteins %	Crude Fat %	Crude Fibre %	Total Carbo- hydrates %	Total Ash %
<b>Varieties—</b>						
T 41	.. 186.4	10.03	4.718	2.16	81.6	1.544
U.S.A. hybrid	.. 181.8	10.18	3.695	1.98	82.4	1.734
T 4111	.. 153.5	10.68	4.673	1.67	81.4	1.601
C.D.	.. ± 6.75	..	±0.137	±0.18	± 0.69	±0.044
<b>Nitrogen—</b>						
0 lb.	.. 164.5	10.33	4.253	1.87	81.8	1.644
40 lb.	.. 170.7	9.93	4.307	1.95	82.2	1.613
80 lb.	.. 186.5	10.51	4.529	1.98	81.4	1.623
C.D.	.. ± 6.75	..	±0.137	..	..	..
<b>Spacing—</b>						
2' × 1'	.. 167.1	10.07	4.306	1.87	82.2	1.612
2' × 2'	.. 176.0	10.12	4.297	1.72	82.2	1.627
3' × 1'	.. 173.3	10.70	4.558	1.97	81.1	1.634
3' × 2'	.. 179.1	10.30	4.290	2.18	81.6	1.632
C.D.	.. ± 7.79	..	±0.158	±0.205	± 0.79	..

and the results of the analysis of variance to judge the significance of the differences observed in Table III.

TABLE III  
*Analysis of Variance. Values for Mean Squares*

Due to	D.F.	Weight 1000 grains	Crude proteins	Crude Fat	Crude Fibre	Total Carbo- hydrates	Total Ash
Varieties	.. 2	3806*	1.3513	4.0163*	0.7345*	3.775‡	0.1139*
Nitrogen	.. 2	1545*	1.2819	0.2563†	0.0370	2.215	0.0030
Spacing	.. 3	228‡	0.7332	0.1529†	0.3124†	2.340‡	0.0009
Varieties × nitrogen	4	165	0.4694	0.0977‡	0.0063	0.760	0.0019
Varieties × spacing	6	83	0.4327	0.0687	0.2178†	1.723	0.0042
Spacing × nitrogen	6	227‡	0.1839	0.1117‡	0.0614	0.583	0.0041
Residual	.. 12	58	0.5264	0.0237	0.0400	0.596	0.0025

\* Shows significance at 0.1% level.

† Shows significance at 1% level.

‡ Shows significance at 5% level.

#### DISCUSSION

From a study of the data presented in Table III it may be seen that the largest amount of effect on the composition of grains has been produced through the three varieties under test by which all the constituents, except crude proteins, have been significantly affected. Next in order comes plant population as affected by spacing although the effects due to spacing have not been so highly significant as those due to varieties. Nitrogen has also produced significant changes in the corn weight and crude fat contents. Effects due to interactions have not been very prominent. It is significant to note that of all the constituents crude proteins have not been affected significantly by the various treatments. This appears to be due to nitrogen having been given as a basal dressing at the rate of 40 lb. nitrogen per acre as farmyard manure.

A careful scrutiny of the mean values for the various constituents as given in Table II brings out the following important facts in regard to the variations in the chemical composition of maize grains as affected by the treatments.

(a) *Corn Weight*.—It can be seen (Table III) that significant effect on corn weight of the grains has been produced due to varieties, nitrogen,

spacing and spacing  $\times$  nitrogen. Amongst the varieties, there has been no significant difference in the corn weight of T 41 and American hybrid although the corn weight of T 4111 was found to be significantly lower than either of the above. Increasing the nitrogen in the fertilizer increased the corn weight. The first addition produced an increase which just touches the level of significance but at 80 lb. of nitrogen per acre the corn weight increased significantly. Similarly, decreasing the plant population showed a tendency of increasing the corn weight slightly. The difference between  $2' \times 1'$  and  $2' \times 2'$  spacings turned out to be highly significant. Nitrogen and spacing produced a significant interaction; highest nitrogen and maximum spacing gave seeds with highest corn weights.

(b) *Crude Proteins*.—There has been no effect on the crude protein contents of the grains and the grains gave a mean crude protein value ranging from 9.93 to 10.70 per cent.

(c) *Crude Fat*.—Varieties have produced the largest amount of difference in regard to the crude fat contents of grains. There was no significant difference between the two Kanpur selections but the U.S.A. hybrid maize contained significantly less crude fat. Increasing the nitrogen dose increased the crude fat content of the grain although at the first dose there was no significant difference over the control. Spacing produced rather an erratic effect for there was no significant difference between the three spacings only  $3' \times 1'$  spacing gave grains with the higher fat content. The interaction between nitrogen and varieties and nitrogen and spacing also came out significant at 5 per cent. level.

(d) *Crude Fibre*.—No significant difference has been observed between the crude fibre content of T 41 and U.S.A. hybrid but T 4111 showed significantly lowest crude fibre content. The crude fibre follows the same trend as the corn weight of the varieties. Spacing also had the effect of increasing the crude fibre with the decrease in plant population. This again shows correlation with the corn weight. Varieties and spacing gave significant interaction.

(e) *Total Carbohydrates*.—Only the effect of varieties and spacing turned out to be significant. U.S.A. hybrid contained significantly more carbohydrates than the Kanpur selections. The effect due to spacing showed that decreasing the plant population tended to decrease the carbohydrate content of grains.

(f) *Minerals*.—In regard to minerals only the varietal differences proved significant. U.S.A. hybrid contained the largest amount of minerals which

was significantly more than either of the two Kanpur selections. Of the Kanpur selections T 41 contained significantly less minerals than T 4111.

#### SUMMARY

1. A study has been made on the chemical composition and quality of maize grains as affected by varieties (T 41, U.S.A. hybrid and T 4111), manures (0, 40 and 80 lb. nitrogen per acre) and spacings or plant population ( $2' \times 1'$ ,  $2' \times 2'$ ,  $3' \times 1'$  and  $3' \times 2'$ ).

2. Representative samples of maize grains from each of the above 36 treatment combinations have been analysed for corn weight, crude proteins, crude fats, crude fibre, total carbohydrates and ash and the data analysed statistically.

3. Largest amount of overall effect on the composition of grains has been produced through varieties. Next in order comes plant population followed by nitrogenous doses.

4. Of all the constituents crude proteins have not been affected by the treatments.

5. Effects of treatments on other constituents have been discussed in the paper.

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# ON A SEQUENCE OF FOURIER COEFFICIENTS

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Let  $f(t)$  be a function which is integrable in the sense of Lebesgue over the interval  $(-\pi, \pi)$  and is defined outside this interval by periodicity. Let the Fourier series of  $f(x)$  be

$$\frac{1}{2} a_0 + \sum_{n=1}^{\infty} (a_n \cos nx + b_n \sin nx) = \frac{1}{2} a_0 + \sum_1^{\infty} A_n(x); \quad (1.1)$$

then the conjugate series of (1.1) is

$$\sum_{n=1}^{\infty} (b_n \cos nx - a_n \sin nx) = \sum_1^{\infty} B_n(x). \quad (1.2)$$

Fejer has shown that if  $l = f(x + 0) - f(x - 0)$  exists and is finite, the sequence  $\{nB_n(x)\}$  is summable  $(C, r)$ ,  $r > 1$ , to the value  $\frac{l}{\pi}$ ; and if  $f$  is of bounded variation, the theorem holds true for  $r > 0$ . It has also been proved that if  $l$  exists and is finite, the sequence  $\{nB_n(x)\}$  is summable by the first logarithmic mean to the same value.

Obrechhoff has shown that if  $f$  is integrable (L) and if  $t^{-1} |f(x+t) - f(x-t) - l|$  is integrable near  $t = 0$ , then  $n^{-1} \sum_1^n r B_r(x) \rightarrow \pi^{-1} l$ .

Mohanty and Nanda have proved that if

$$f(x+t) - f(x-t) - l = O \left\{ \left( \log \frac{1}{t} \right)^{-1} \right\} \text{ as } t \rightarrow 0$$

and  $a_n$  and  $b_n$  are  $O(n^{-\delta})$ ,  $0 < \delta < 1$ ,

then the sequence  $\{nB_n(x)\}$  is summable  $(C, 1)$  to the value  $\frac{l}{\pi}$ . From this result they have deduced the Hardy and Littlewood's test for convergence of the conjugate series (1.2) by applying a Tauberian Theorem of Hardy and Littlewood.

We write

$$\psi(t) = f(x+t) - f(x-t) - l$$

The object of this note is to prove the following theorem:—

THEOREM 1. *If*

$$\Psi(t) = \int_0^t \psi(u) du = O(t) \quad (1.3)$$

and

$$\int_{\epsilon}^{\delta} \frac{|\psi(t + \epsilon) - \psi(t)|}{t} dt \rightarrow 0, \quad (1.4)$$

for some fixed  $\delta$ , when  $\epsilon \rightarrow +0$ , then the sequence  $\{nB_n(x)\}$  is summable  $(C, 1)$  to the value  $\frac{l}{\pi}$ .

From Theorem 1, we deduce Lebesgue's test for convergence of the conjugate series (1.2) by applying Tauber's Second Theorem.

It is to be noted that Obrechhoff has proved the summability  $(C, 1)$  of the sequence  $\{nB_n(x)\}$  under Dini's criterion for the convergence of Fourier series while from Fejer's result it is obvious that the sequence is also summable  $(C, 1)$  under Jordan's criterion. In Theorem 1, we prove the same result under Lebesgue's criterion. As it is well known that Lebesgue's test includes both Dini's and Jordan's tests, Theorem 1 includes the above-mentioned results of Obrechhoff and Fejer.

# STUDY IN THE PRECIPITATION OF YELLOW AND BROWN HYDROUS FERRIC OXIDE

## Part XI. Electron Diffraction Study of the Different Samples of Hydrous Ferric Oxide

BY SITA RAM GUPTA AND SATYESHWAR GHOSH

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IN a series of our papers<sup>1</sup> we have reported the precipitation of hydrous ferric oxide in two stages leading to the formation of a yellow variety of hydrous ferric oxide. If a very deficient amount of an alkali solution is added to a ferric chloride solution in presence of some bivalent anion-like  $\text{SO}_4^{--}$  or  $\text{Cr}_2\text{O}_7^{--}$ , etc., a brown precipitate immediately separates and after a certain time lag (depending upon the amount of alkali added) a slow precipitation of yellow hydrous ferric oxide free from any  $\text{Cl}^-$  ions and associated with negligible amount of  $\text{SO}_4^{--}$  ions takes place. The brown and the yellow hydrous ferric oxides thus obtained differ remarkably in their chemical and physical properties from the well-known  $\alpha$ -variety of brown hydrous ferric oxide, obtained by the interaction of equivalent amount of a soluble ferric salt solution and an alkali solution. These samples are very inactive as compared to  $\alpha$ -variety in their solubility,<sup>2</sup> adsorptive capacity,<sup>3</sup> catalytic active towards the decomposition of hydrogen peroxide<sup>4</sup> and magnetic properties.<sup>5</sup> However, these samples resemble in their inertness to the yellow  $\gamma$ -variety and  $\beta$ -variety of hydrous ferric oxide as described by Welo and Baudish<sup>6</sup> and Weiser<sup>7</sup> respectively. It became therefore necessary to make some electron diffraction study to see whether these samples are crystalline or amorphous and whether it is  $\gamma$ - or  $\beta$ -variety or altogether different.

For electron diffraction study the following samples were prepared as described in our earlier papers (*loc. cit.*) and the composition of the reacting solutions may be given below.

TABLE I

Sample	Volume of 0.3N NaOH c.c.	Volume of 0.1M $\text{FeCl}_3$ c.c.	Volume of 0.5M $\text{K}_2\text{SO}_4$ c.c.	Volume of Water c.c.
1—Y	100	1000	200	700
4—Y	400	1000	200	400
4—B	400	1000	200	400
B—E <sub>a</sub>	1000	1000	..	..

The samples after their preparation were washed to free them from any associated electrolyte, in a special apparatus operated by a current of distilled water. These samples were then dried at room temperature and also a portion of the dried sample was heated in a crucible to dehydrate them. It is very interesting to see that the yellow sample becomes red on heating. The details of the electron diffraction experiment are given below.

The ferric oxide samples were studied in the hot cathode electron diffraction camera (modified Finch type) at 40 K.V.

#### PREPARATION OF THE SPECIMEN

The samples were finely powdered in an agate mortar and then dusted on a thin collodion film on a glass slide while still wet. The film when dry was taken out, mounted on a nickel gauze and then studied in the electron diffraction camera.

The following are the results:

TABLE II  
*Sample B—E<sub>q</sub>*  
(Fig. 2)

Intensity	$d$ in Å ( $hkl$ )
<i>s</i>	1.92
<i>s</i>	1.55
<i>m</i>	1.21

TABLE III  
*Sample 4—B*  
(Fig. 1)

Intensity	$d$ in Å ( $hkl$ )	Intensity	$d$ in Å ( $hkl$ )
<i>vf</i>	4.84	<i>s</i>	1.93
* <i>s</i>	4.18	<i>vf</i>	1.82
* <i>f</i>	3.64	<i>s</i>	1.54
<i>vf</i>	3.04	<i>f</i>	1.34
* <i>m</i>	2.88	<i>m</i>	1.22

TABLE IV  
*Sample 1—Y heated*  
 (Fig. 3)

Intensity	$\frac{d \text{ in } \text{\AA}}{(hkl)}$	Intensity	$\frac{d \text{ in } \text{\AA}}{(hkl)}$
*s	4.18	v.s.	1.58
*f	3.60	f	1.54
vf	3.20	f	1.37
vf	3.00	f	1.34
s	2.74	vf	1.27
m	2.58	vf	1.18
m	2.56	diffuse	1.02
f	2.41	f	0.91
vf	2.14	vf	0.77
vf	1.98		

N.B.—s, Strong; v, Very; m, Medium; f, Faint.

\* Due to paraffin wax.

The asterics are due to the diffractions of paraffin wax, which contaminated the samples. The diffraction patterns of paraffin wax was taken as an internal standard assuming that (110) spacing of the wax to be 4.18 Å. The above diffraction patterns show that the samples 4—B, B—E<sub>q</sub> and 1—Y (heated) are crystalline in nature. Moreover the samples 4—B and B—E<sub>q</sub> as seen from the Figs. 1 and 2 have the similar structure and the same spacing and hence they are identical compounds and sample 1—Y (heated) is different.

The yellow dried samples 4—Y and 1—Y did not yield any clear diffraction patterns other than the amorphous pattern of the base collodion. This may be that these samples are amorphous, but when heated yield the diffraction pattern as shown in Fig. 3.

It is very interesting to note here that Weiser (*loc. cit.*) made an X-ray study of the yellow  $\beta$ -hydrous ferric oxide and found it to be crystalline. Similarly Albrecht<sup>8</sup> from X-ray study and Fricke and Schoon<sup>9</sup> from the electron diffraction studies concluded that the yellow  $\gamma$ -hydrous ferric oxide is crystalline in nature. It therefore appears that the yellow sample of hydrous ferric oxide described here is another modification.

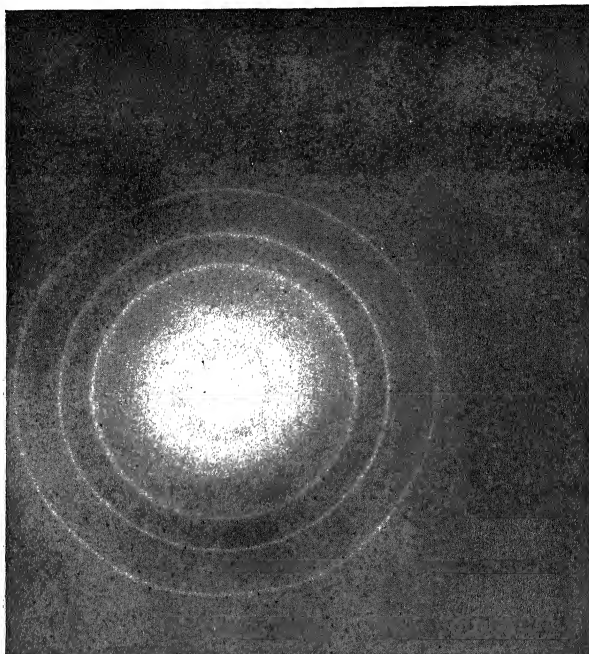


FIG. 1. 4—B (A 59)

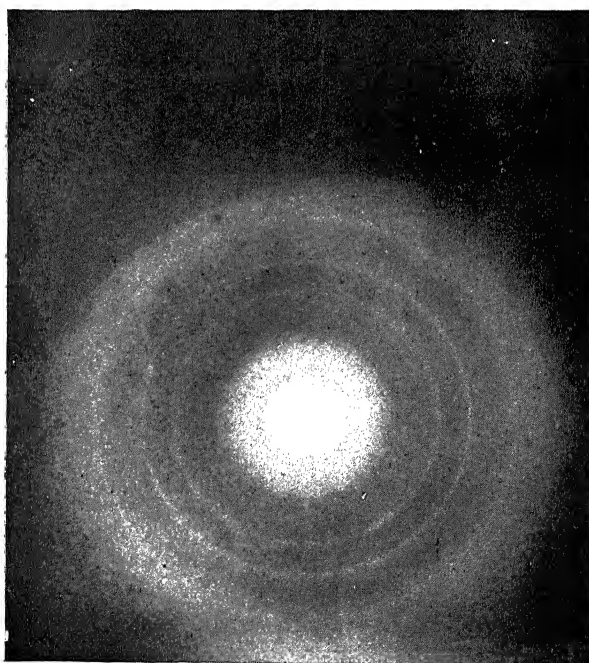


FIG. 2. B—Eq. (A 53)

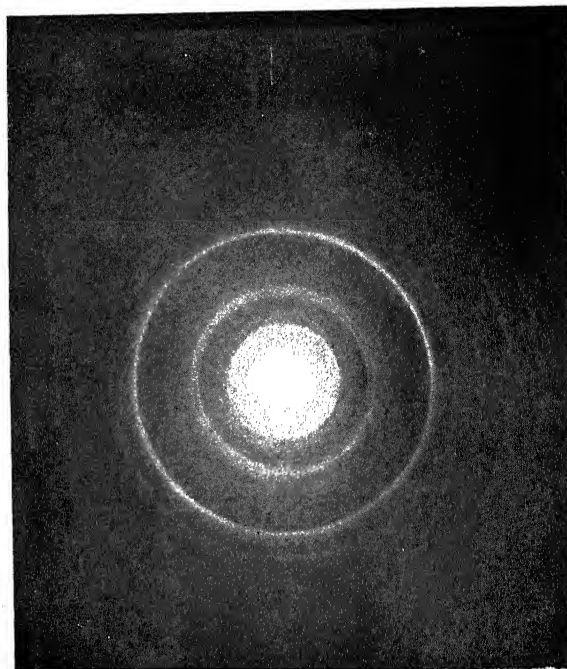


FIG. 3. 1—Y (heated) ( $\lambda$  64)

The X-ray study of these samples is in progress.

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# STUDIES IN THE CHANGE OF THE HYDROGEN-ION CONCENTRATION OF COLLOIDAL HYDROUS OXIDE SOLS DURING THEIR COAGULATION

## Part I. Coagulation of Ferric Oxide Sols by Potassium Sulphate

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IN order to elucidate the mechanism of coagulation of sols by electrolytes, Weiser and co-workers<sup>1-2</sup> have carried out extensive work on the exchange of adsorption, specially of anions, during the coagulation of a hydrous oxide sol by potassium sulphate. Similar work has been undertaken by other workers<sup>3</sup> also, and in general it has been shown that more chloride becomes osmotically active from the sols of hydrous oxides of iron, aluminium and chromium, obtained either by peptizing them with hydrochloric acid, or from the chlorides of these metals by the addition of ammonium carbonate. These observations lead to the conclusion that the chloride ions are also bound up with the micellé or the double layer of the charged colloidal particles, and that these ions, which are not ordinarily osmotically active are liberated out by the addition of a polyvalent ion like sulphate, and it is less prominent with univalent coagulating ions.

It is well known that hydroxonium ions have great influence on the stability of such hydrous oxides as those of iron, aluminium and chromium. In order to get a fuller picture of the mechanism of coagulation, we have studied in this paper the influence played by the precipitating ion sulphate on the amount of hydroxonium ion present in the sols of hydrous ferric oxide.

### EXPERIMENTAL

Two samples of sols of ferric hydroxide were prepared, one by adding ammonium carbonate to a strong ferric chloride solution and the other by peptizing a freshly precipitated hydrous oxide sample by hydrochloric acid, both at the room temperature (about 25° C.). The sols thus obtained were dialysed at the room temperature and were analysed at different stages of purity. The ferric oxide content and the chloride ion content as impurity in the sample were determined by dissolving a known quantity of the sol in nitric acid and by estimating both iron and chloride ions by usual volumetric methods. A known volume of the sol of known purity was taken out and

was treated by different amount of potassium sulphate, below and above the precipitating concentration and were made upto a constant volume by the addition of water. The variations in the hydroxonium ion concentration were determined with the help of a glass electrode by means of an A.C. operated L. & N. pH indicator. Each reading was recorded after a lapse of half an hour.

One of the typical results with a sample of a sol of hydrous ferric oxide obtained by peptizing by hydrochloric acid is reproduced in Table I.

TABLE I

Concentration:  $\text{Fe}_2\text{O}_3$  = 6.32 gm./litre.  
 $\text{Cl}^-$  = 0.2766 gm./litre.  
 Purity:  $\text{Fe}_2\text{O}_3/\text{Cl}^-$  = 22.9

Concentration of the electrolyte for the coagulation of 10 c.c. of the sol. = 4.8 c.c. N/125 potassium sulphate.

Sol in ml.	Water in ml.	$\text{K}_2\text{SO}_4$ N/125 in ml.	pH
10	10	0.0	4.80
10	9.5	0.5	4.84
10	9.0	1.0	4.98
10	8.5	1.5	5.20
10	8.0	2.0	5.35
10	7.5	2.5	5.50
10	7.0	3.0	5.90
10	6.5	3.5	6.30
10	6.0	4.0	6.50
10	5.5	4.5	6.60
10	5.0	5.0	6.70
10	4.5	5.5	6.80
10	4.0	6.0	6.88
10	3.5	6.5	6.95
10	3.0	7.0	6.98
10	2.5	7.5	7.00
10	2.0	8.0	7.00
10	1.5	8.5	7.00
10	1.0	9.0	7.00
10	0.5	9.5	7.00
10	0.0	10	7.00

These results are plotted in Fig. 1 for the variation of pH effected by the addition of different quantity of N/125 potassium sulphate. Similar results

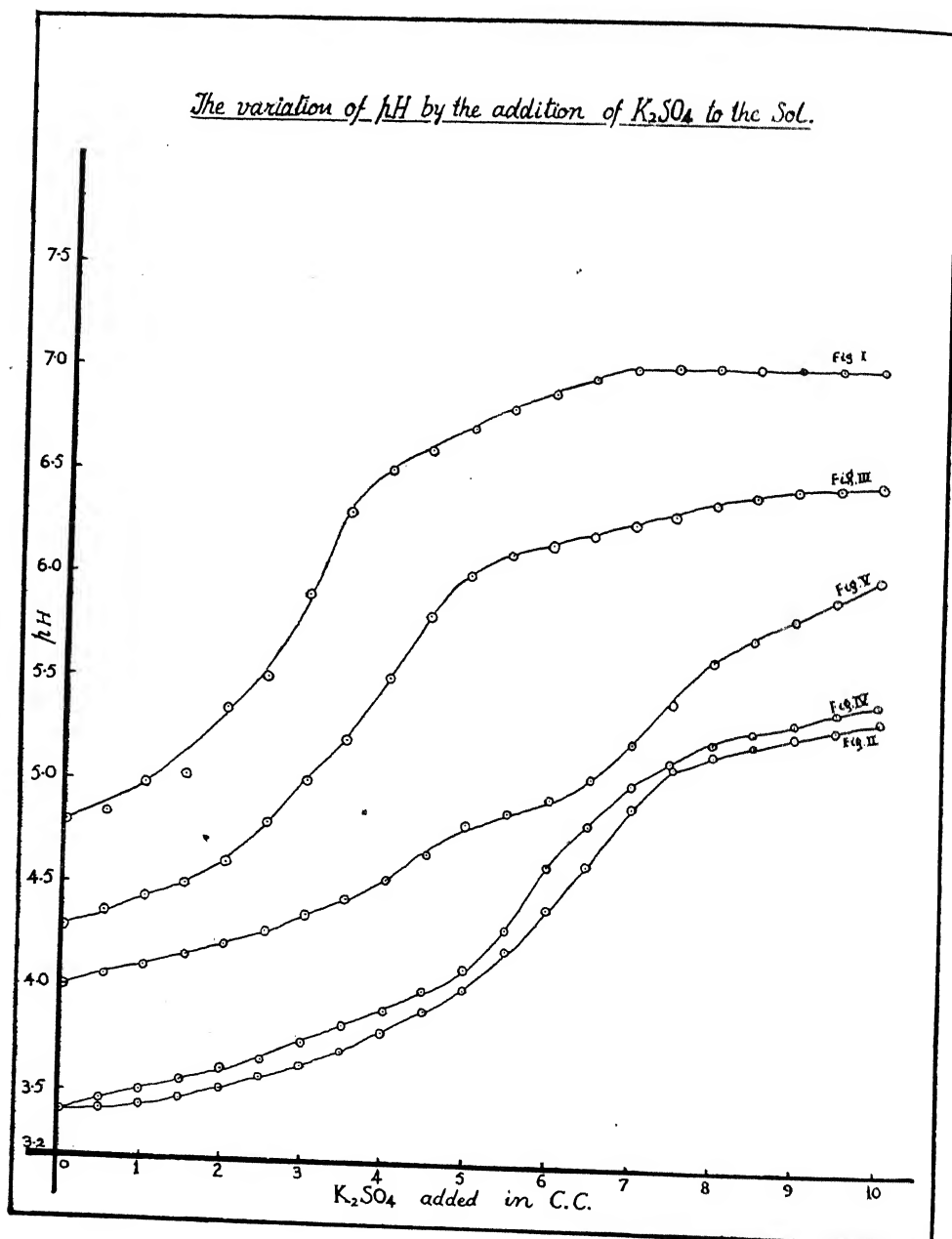


FIG. 1

were obtained for other sols of different purities and the methods of preparation, and these results are given in Tables II to V, and are plotted in Figs. 2 to 5.

TABLE II

Concentration:  $\text{Fe}_2\text{O}_3$  = 7.346 gm./litre. $\text{Cl}^-$  = 0.114 gm./litre.Purity:  $\text{Fe}_2\text{O}_3/\text{Cl}^-$  = 64.4.

Concentration of the electrolyte for the coagulation of 10 c.c. of the sol = 5.6 c.c., N/125 potassium sulphate. Sol prepared by peptizing hydrous oxide by HCl.

Sol in ml.	Water in ml.	$\text{K}_2\text{SO}_4$ N/125 in ml.	pH
10	10	0.0	3.40
10	9.5	0.5	3.41
10	9.0	1.0	3.43
10	8.5	1.5	3.48
10	8.0	2.0	3.52
10	7.5	2.5	3.58
10	7.0	3.0	3.62
10	6.5	3.5	3.70
10	6.0	4.0	3.80
10	5.5	4.5	3.90
10	5.0	5.0	4.00
10	4.5	5.5	4.20
10	4.0	6.0	4.40
10	3.5	6.5	4.60
10	3.0	7.0	4.90
10	2.5	7.5	5.10
10	2.0	8.0	5.15
10	1.5	8.5	5.20
10	1.0	9.0	5.25
10	0.5	9.5	5.28
10	0.0	10	5.32

TABLE III

Concentration:  $\text{Fe}_2\text{O}_3$  = 6.256 gm./litre. $\text{Cl}^-$  = 0.081 gm./litre.Purity:  $\text{Fe}_2\text{O}_3/\text{Cl}^-$  = 77.2.

Concentration of the electrolyte for the coagulation of 10 c.c. of the sol = 4.6 c.c. N/300 potassium sulphate. Sol prepared by peptizing hydrous oxide by HCl.

Sol in ml.	Water in ml.	$\text{K}_2\text{SO}_4$ N/300 in ml.	pH
10	10	0.0	4.30
10	9.5	0.5	5.36
10	9.0	1.0	4.44
10	8.5	1.5	4.50
10	8.0	2.0	4.60
10	7.5	2.5	4.80
10	7.0	3.0	5.00
10	6.5	3.5	5.20
10	6.0	4.0	5.50
10	5.5	4.5	5.80
10	5.0	5.0	6.00
10	4.5	5.5	6.10
10	4.0	6.0	6.15
10	3.5	6.5	6.20
10	3.0	7.0	6.25
10	2.5	7.5	6.30
10	2.0	8.0	6.35
10	1.5	8.5	6.39
10	1.0	9.0	6.41
10	0.5	9.5	6.43
10	0.0	10	6.45

TABLE IV

Concentration:  $\text{Fe}_2\text{O}_3$  = 12.320 gm./litre.

$\text{Cl}^-$  = 0.97 gm./litre.

Purity:  $\text{Fe}_2\text{O}_3/\text{Cl}^-$  = 12.7.

Concentration of the electrolyte for the coagulation of 10 c.c. of the sol = 6.3 c.c. N/50 potassium sulphate. Sol prepared by ammonium carbonate.

Sol in ml.	Water in ml.	$\text{K}_2\text{SO}_4$ N/50 in ml.	pH
10	10	0.0	3.40
10	9.5	0.5	3.45
10	9.0	1.0	3.50
10	8.5	1.5	3.55
10	8.0	2.0	3.60
10	7.5	2.5	3.65
10	7.0	3.0	3.74
10	6.5	3.5	3.82
10	6.0	4.0	3.90
10	5.5	4.5	4.00
10	5.0	5.0	4.10
10	4.5	5.5	4.30
10	4.0	6.0	4.60
10	3.5	6.5	4.80
10	3.0	7.0	5.00
10	2.5	7.5	5.10
10	2.0	8.0	5.20
10	1.5	8.5	5.25
10	1.0	9.0	5.30
10	0.5	9.5	5.35
10	0.0	10	5.40

TABLE V

Concentration:  $\text{Fe}_2\text{O}_3$  = 11.04 gm./litre. $\text{Cl}^-$  = 0.66 gm./litre.Purity:  $\text{Fe}_2\text{O}_3/\text{Cl}^-$  = 16.7.

Concentration of the electrolyte for the coagulation of 10 c.c. of the sol = 6.4 c.c. N/100 potassium sulphate. Sol prepared by ammonium carbonate.

Sol in ml.	Water in ml.	$\text{K}_2\text{SO}_4$ N/50 in ml.	pH
10	1.0	0.0	4.00
10	9.5	0.5	4.05
10	9.0	1.0	4.10
10	8.5	1.5	4.15
10	8.0	2.0	4.20
10	7.5	2.5	4.27
10	7.0	3.0	4.35
10	6.5	3.5	4.43
10	6.0	4.0	4.52
10	5.5	4.5	4.65
10	5.0	5.0	4.80
10	4.5	5.5	4.86
10	4.0	6.0	4.93
10	3.5	6.5	5.03
10	3.0	7.0	5.20
10	2.5	7.5	5.40
10	2.0	8.0	5.60
10	1.5	8.5	5.70
10	1.0	9.0	5.80
10	0.5	9.5	5.90
10	0.0	10	6.00

## DISCUSSION

A perusal of the experimental data presented here shows that in all cases the addition of  $K_2SO_4$  to a hydrous ferric oxide sol increases the pH value, showing that alkali is generated in the process, so much so that a sol, which is distinctly acidic, becomes neutral. The increase in the alkalinity is not only limited upto the coagulation point, but it goes on increasing even beyond it. Further it is interesting to find that the curves are in general S-shaped which becomes asymptotic after the amount of potassium sulphate added exceeds its precipitation value.

It will be of interest to recall here that Weiser and Co-workers<sup>4-5</sup> in their investigations on hydrous aluminium oxide reported a very slight increase in acidity by the addition of potassium sulphate to the sol. On the other hand Thomas<sup>6</sup> has reported for the sol of the same hydrous oxide an increase of pH value by the addition of potassium sulphate. Similarly Weiser<sup>7</sup> has reported for the sol of chromium hydroxide acidified with hydrochloric acid that the pH value increased by the addition of a coagulating electrolyte like potassium sulphate.

In a number of publications Thomas and Co-workers<sup>6</sup> suggested that the colloidal micelles of iron, aluminium and chromium oxide peptized with hydrochloric acid consists of olated and possibly oxolated oxychloride complexes of the Werner type as formulated by Bjerrum; so that the increased pH value noted for these sols by the addition of neutral electrolytes could be attributed to the replacement of the OH groups by the anion of the added salt from the colloidal micelle. Bohm,<sup>8</sup> however, showed as early as 1924, by X-ray analysis that hydrous aluminium oxide sol obtained from aluminium acetate is not a basic salt of Werner complex. Moreover, it is well known that the adsorption of coagulating ions is more in acid solutions than in neutral ones. If any basic salts are formed, the probability is more in the acid solutions, and necessarily smaller amounts of the anions could possibly enter the colloidal micelle in place of OH groups present therein. But the fact remains that alkalinity is developed by the stepwise addition of potassium sulphate to the sols of such hydrous oxides and hydrated ferric oxide as investigated in this paper.

In a number of publications from these laboratories<sup>9</sup> on the precipitation of hydrous oxides it has been reported that slightly smaller quantity than the equivalent amount of alkali completely precipitates the hydrous oxide from the soluble salts of these metals. It has also been shown that there is no evidence of the formation of basic salt at any stage, specially in the precipitation of hydrous oxides, as iron, aluminium and chromium. The

completion of the precipitation has been ascribed to the generation of alkali produced by the action of the neutral salts generated as a product of the reaction. We are inclined to believe that alkali is generated by the adsorption of the hydrogen-ions by the discharged colloidal matter by the anion of the neutral salts.

We shall now picture the neutralization of electric charge on the subsequent coagulation of a sol of hydrous ferric oxide. When potassium sulphate is added to the sol, the sulphate ion neutralizes the positive charge on the colloidal particles to its critical potential. Where aggregation is favoured, in this operation, the osmotically inactive chloride ions are set free, but this does not explain the generation of alkalinity in the system. We, therefore, suggest that the mechanism of coagulation involves the lowering of electrical charge on the colloidal surface, which necessarily increases the adsorption of hydroxonium ion by the partially neutralized colloidal surface. Thus the alkalinity of the system is increased and with consequent increase in the electric charge on the colloidal surface, which is again decreased by the sulphate ions, which are present in the system as excess. This process continues till the adsorption of the sulphate ion overcomes any increase of electric charge by the adsorption of the hydroxonium ions.

It is quite well known that coagulation is effected long before complete neutralization and the process continues beyond the coagulation point. Since the surface of the adsorbing material is quickly decreased after coagulation the nature of the curves in the increase of pH shows a variation.

Further work is in progress in this laboratory in order to elucidate the mechanism of coagulation from these points of view.

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